AD_____

Award Number: W81XWh-05-1-0250

TITLE: Interaction of AIB1 and BRCA1 in the Development of Breast Cancer

PRINCIPAL INVESTIGATOR: John T. Lahusen

CONTRACTING ORGANIZATION: Georgetown University
Washington, DC 20057

REPORT DATE: March 2008

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
20-03-2008	ANNUAL SUMMARY	21 FEB 2005 - 20 FEB 2008

4. TITLE AND SUBTITLE
Interaction of AIB1 and BRCA1 in the Development of Breast Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-05-1-0250

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
John T. Lahusen

Email: jtl5@georgetown.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Georgetown University
Washington, DC 20057

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

AIB1 (SRC3) belongs to the p160 family of steroid receptor coactivators including SRC-1 and SRC-2. AIB1 interacts with several nuclear receptors including estrogen and progesterone receptors in a ligand-dependent manner and enhances their transcriptional activity. AIB1 is amplified and/or overexpressed in approximately 30% of breast cancers and can increase the sensitivity of breast cancer cells to estrogen and to growth factor signaling. BRCA1 regulates cell cycle progression, apoptosis induction, transcription, and DNA repair. From 5-10% of total breast cancers are due to germ-line BRCA1 mutations that lead to a deficiency in the BRCA1 protein. We have observed that AIB1 can partially reverse BRCA1 mediated repression of ER-dependent transcriptional activity in breast cancer. This research will identify if there is a functional consequence of an interaction between AIB1 and BRCA1 in breast cancer.

15. SUBJECT TERMS

None listed.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	29	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

INTRODUCTION

The *AIB1* gene was initially identified to be amplified on a portion of human chromosome 20q that is frequently amplified in breast cancer (1). AIB1 (SRC-3) was later characterized as a nuclear receptor coactivator that belongs to the p160 family of steroid receptor coactivators including SRC-1 and SRC-2. Steroid receptor coactivators recruit other coactivators and the basal transcriptional machinery to nuclear receptors. AIB1 interacts with nuclear receptors including estrogen and progesterone receptors in ligand-dependent manner and enhances their transcriptional activity. The *AIB1* gene is amplified in 5-10% of human breast tumors and the mRNA is overexpressed in 31-64% of breast tumors (1-3). An isoform of AIB1, AIB1-Δ3, encodes a 130 kDa protein that lacks the amino-terminal bHLH/PAS dimerization domain and is a more active transcriptional coactivator of ERα and PR than full-length AIB1 (4). Also, AIB1 levels are limiting for both hormone (5-8) and insulin-like growth factor I (IGF-I) signaling responses in mammary cancer cells *in vitro* and for H-Ras-induced mammary tumorigenesis in mice (8).

Germ-line mutations of the breast cancer susceptibility gene *BRCA1* account for 5-10% of breast cancers. BRCA1 is involved in DNA repair, progression through the cell cycle, apoptosis, maintenance of DNA integrity, and regulation of transcription. BRCA1 has been shown to physically associate with transcriptional activators, repressors, DNA-binding transcription factors, and DNA repair factors. It has been reported that BRCA1 suppresses the transcriptional activity of estrogen and progesterone receptor (9, 10). The lack of wild-type *BRCA1* expression in mammary epithelium may lead to increased DNA damage and cellular proliferation. BRCA1 may suppress the proliferation of mammary epithelial cells by estrogen. Thus, the loss of the inhibitory activity of BRCA1 may lead to mammary carcinogenesis. It has been reported that BRCA1 interacts with SRC-1 (11), but there are no reports of an interaction of

AIB1 with BRCA1. Preliminary data in our lab showed that AIB1 and BRCA1 proteins interact in MCF-7 breast cancer cells. Since it has been reported that BRCA1 suppresses ERα activity in breast cancer cells, I assessed whether overexpression of AIB1 could reverse this effect. Also, preliminary evidence suggests that AIB1 can partially reverse the BRCA1-induced suppression of ERα transcriptional activity. We propose that the ratio of BRCA1 and AIB1 in the cell determines the response to estrogen. It could be that high BRCA1 levels in the cell leads to inhibition of AIB1's coactivator ability and decreased ERα activity, but when AIB1 is overexpressed or levels of BRCA1 decrease then AIB1 is free to coactivate ERα transcriptional activity. Increased estrogen-induced gene expression can lead to over-proliferation of breast epithelial tissue and eventually tumorigenesis. I want to determine if there is a functional consequence of AIB1 and BRCA1 in breast cancer in relation to hormone and growth factor signaling.

BODY

This final report will address the progress for fulfilling the aims as outlined in **Task 1** and **Task 2** of the **Statement of Work** for grant W81XWh-05-1-0250. Section (a) of **Task 1** is to determine if AIB1 and BRCA1 protein interact directly in breast cancer cells and then define the region/s necessary for interaction with BRCA1. Preliminary experiments showed that AIB1 and BRCA1 protein interacted by co-immunoprecipitation using MCF-7 cellular lysate (Figure 1). In this experiment, BRCA1 was immunoprecipitated with a BRCA1 antibody (Ab2, Oncogene Science) and GammaBind Sepharose beads (GE Healthcare). AIB1 was detected by immunoblot with an anti-AIB1 antibody (BD Transduction). There was increased association of AIB1 with BRCA1 in this experiment and other similar experiments (Figure 1A). However, experiments using a negative control (mouse IgG) and GammaBind

Sepharose beads blocked with bovine serum albumin suggested that a portion of the interaction was not specific because AIB1 was detected in the mouse IgG immunoprecipitates (Figure 1B). Another experiment was performed in 293T kidney cells, which are highly transfectable. 293T cells were transfected with Flag-epitope-tagged AIB1 and AIB1-Δ3 constructs using Fugene6. Lysates were immunoprecipitated with anti-Flag and immunoblotted for AIB1, BRCA1, and p300. The expression of AIB1 and AIB1-Δ3 was increased as indicated by the AIB1 immunoblot of the Flag immunoprecipitates (Figure 2). Interestingly, as indicated from the input samples, transfection of AIB1 and AIB1-Δ3 increased BRCA1 protein expression, thus suggesting that AIB1 may regulate BRCA1 expression in cells (Figure 2). BRCA1 was detected in the input samples, but there was no BRCA1 detected in the Flag immunoprecipitates (Figure 2). However, the p300 protein, which has been shown to interact directly with AIB1, was detected in the Flag-AIB1 immunoprecipitate (Figure 2). Therefore, AIB1 could interact with p300 but not BRCA1 in this experiment. The experiments so far for section (a) of **Task 1** suggest that AIB1 does not interact directly with BRCA1 as a result of non-specific interaction of AIB1 with the IgG immunoprecipitates as observed in some experiments.

As observed in Figure 2, overexpression of AIB1 and AIB1-Δ3 in 293T cells results in increased expression of BRCA1 protein expression. Regulation of BRCA1 levels by AIB1 was also verified in MCF-7 cells. As a result of small-interfering RNA (siRNA)-mediated reduction of AIB1 in MCF-7 cells, BRCA1 protein expression was decreased (Figure 3). Therefore, BRCA1 protein expression is regulated by both AIB1 overexpression and knockdown.

Section (b) of **Task 1** is to determine if overexpression of BRCA1 in breast cancer cells is able to suppress the coactivator potential of AIB1 but not AIB1(BRCA1-) that does not bind to BRCA1. BRCA1 has been shown to suppress estrogen-dependent transcription in breast cancer cells (9). As part of the **Statement of Work**, it was determined if AIB1 can reverse BRCA1-dependent suppression of ERα transcriptional activity. Preliminary data using MCF-7 breast

cancer cells indicated that BRCA1 suppressed estrogen-stimulated estrogen receptor transcriptional activity and AIB1 could partially reverse this effect (Figure 4). However, these results were not consistently reproducible in follow-up experiments.

For **Task 2**, it was determined if AIB1 and potentially if AIB1(BRCA1-) has any effect on IGF-1 or EGF-dependent biological responses, signaling, and gene expression. IGF-1 was not a strong inducer of cyclin D1 promoter activity. Therefore, HCC1937 (BRCA1-deficient) and MCF-7 (BRCA1-wild type) breast cancer cells were tested for induction of cyclin D1 promoter activity by EGF and heregulin-β. Heregulin-β was a stronger inducer of cyclin D1 promoter activity in these cell lines. In HCC1937 and MCF-7 cells, induction of -1745 cyclin D1 promoter activity by heregulin-β was enhanced with overexpression of AIB1-Δ3 (Figure 3A and 3B). This suggests that enhancement of cyclin D1 promoter activity by AIB-Δ3 is independent of the status of BRCA1 in the cell.

Fulfilling the aims of **Task 2** was dependent on identifying a region of AIB1 that interacts with BRCA1. However, the ability of AIB1 to interact directly with BRCA1 was inconclusive. Therefore, it was necessary to compare EGF-dependent phenotypic effects in both BRCA-wild type and BRCA1-deficient breast cancer cells. It was determined if the expression level of AIB1 or BRCA1 affects the ability of cells to proliferate in response to EGF. MDA-MB-231 (BRCA1-wild type) breast cancer cells were tested because they proliferate in response to EGF. Initially, MDA-MB-231 cells were treated with AIB1 siRNA and then stimulated with EGF for 72 hours (Figure 6). A reduction of AIB1 protein levels in MDA-MB-231 cells with AIB1 siRNA resulted in a significant inhibition of EGF-stimulated proliferation in comparison to control siRNA treated cells (Figure 6). Therefore, this demonstrates that cellular AIB1 levels are limiting for EGF-stimulated proliferation of breast cancer cells. It still remains to be determined if there is a role for BRCA1 in EGF-stimulated proliferation of breast cancer cells. Since it was

observed that there was a reduction in EGF-stimulated proliferation of AIB1 siRNA treated cells, it was determined if AIB1 knockdown affected EGFR levels or its tyrosine phosphorylation. Ligand-bound EGFR results in activation of tyrosine kinase activity and tyrosine phosphorylation of multiple intracellular tyrosine residues (Figure 7). As a result of AIB1 knockdown in MDA-MB-231 breast cancer cells, there was no change in EGFR levels (Figure 8A). However, there was a significant decrease in overall EGF-induced tyrosine phosphorylation of EGFR as detected with a phosphotyrosine antibody (Figure 8A). This result was then verified with antibodies against tyrosine phosphorylated residues of EGFR. Treatment of MDA-MB-231 cells with AIB1 siRNA resulted in decreased EGF-induced phosphorylation of EGFR on multiple tyrosine residues (Figure 8B). However, in BRCA1-deficient HCC1937 breast cancer cells, EGF-induced EGFR tyrosine phosphorylation was not affected by reduced AIB1 expression (Figure 9). It still remains to be determined if this is a result of BRCA1 deficiency in HCC1937 cells. Interestingly, it was previously shown that BRCA1 is phosphorylated through growth factor signaling (12). It is possible that AIB1 could regulate BRCA1 function through control of EGFR signaling. To determine the specificity of AIB1 knockdown on EGFR phosphorylation, it was tested whether reduced AIB1 expression affected the phosphorylation of the EGFR family member HER3. HER3 is tyrosine phosphorylated as a result of heregulin-β-induced heterodimerization with HER2. SK-BR-3 breast cancer cells express EGFR, HER2, and HER3. Similarly with MDA-MB-231 cells, a reduction of AIB1 in SK-BR-3 cells results in decreased EGF-induced EGFR tyrosine phosphorylation (Figure 10A). However, heregulin-β-induced tyrosine phosphorylation of HER3 was unchanged (Figure 10B).

Binding of EGF to EGFR results in recruitment of proteins to EGFR that are involved in activation of downstream signaling molecules (Figure 7). Therefore, it was determined if decreased EGFR tyrosine phosphorylation as a result of AIB1 knockdown affected the interaction of proteins with EGFR. Treatment of MDA-MB-231 cells with AIB1 siRNA resulted

in decreased EGF-induced interaction of Shc, GRB2, and Cbl with EGFR (Figure 11). A reduction in AIB1 levels also resulted in decreased EGF-induced activation of signaling pathways downstream of EGFR. A reduction in AIB1 levels in MDA-MB-231 cells resulted in decreased EGF-induced phosphorylation of ERK1/2 and STAT5 but not AKT (Figure 12).

There are several possible mechanisms of how AIB1 levels could affect EGFR tyrosine phosphorylation. EGFR tyrosine phosphorylation can be regulated through changes in ligand-binding affinity, by an increase in the inhibitory phosphorylation of serine and threonine residues, and by removal of phosphate groups on tyrosine residues by tyrosine phosphatases (Figure 13)(13-15). AIB1 knockdown did not affect the number of EGF receptors on the cell surface or the number of receptors internalized as a result of EGF stimulation (Figure 14). Then it was tested whether AIB1 knockdown increased the expression and/or activity of a tyrosine phosphatase. A reduction in AIB1 did not affect the expression of SHP-1 or PTP-1B, tyrosine phosphatases that have previously be shown to dephosphorylate EGFR (Figure 15A)(15, 16). Next, to determine if a knockdown in AIB1 affected tyrosine kinase activity, MDA-MB-231 cells were treated with the tyrosine phosphatase inhibitor vanadate. The induction of EGFR phosphorylation by EGF was greater in AIB1 siRNA-treated cells with vanadate than control-siRNA treated cells (Figure 15B). This suggests that AIB1 regulates the activity of a tyrosine phosphatase that targets EGFR for dephosphorylation.

Figure 1

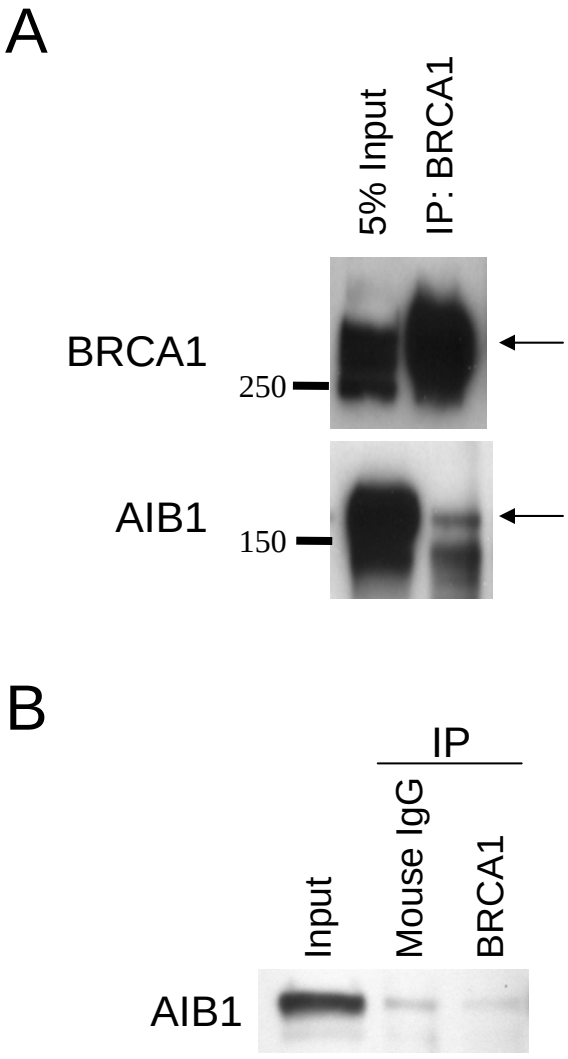


Figure 1. **Co-immunoprecipitation of AIB1 with BRCA1 in MCF-7 breast cancer cells**.
(A & B) Whole cell lysate was immunoprecipitated with a monoclonal BRCA1 antibody (AB2, Oncogene Science) and (B) a mouse IgG. then BRCA1 and AIB1 were detected with anti-BRCA1 and anti-AIB1 (BD Transduction) antibodies.

Figure 2

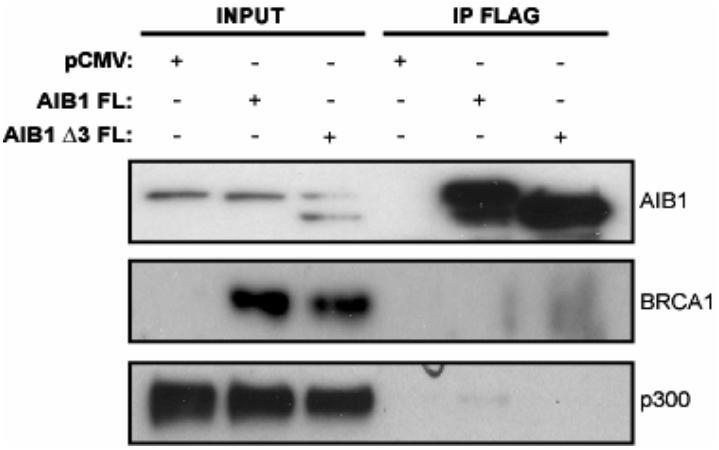


Figure 2. **Determine binding of BRCA1 as a result of overexpression of AIB1 and AIB1-Δ3 in 293T cells.**
293T human kidney cells were transfected for 24 hrs with either pcDNA3, AIB1-Flag, or AIB1-Δ3-Flag plasmid using Fugene 6 (Roche). Whole cell lysates were immunoprecipitated with anti-Flag (M2, Sigma) and then immunoblotted with antibodies for either AIB1, BRCA1, or p300.

Figure 3

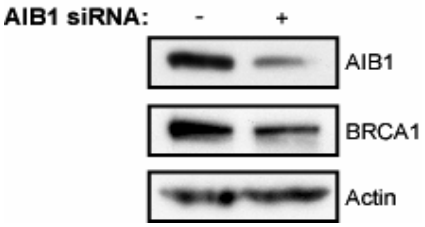


Figure 3. Regulation of BRCA1 protein levels by AIB1 in MCF-7 breast cancer cells.
MCF-7 cells were transfected with either control or AIB1 small-interfering RNA (siRNA) for 24 hrs with Lipofectamine 2000. Whole cell lysates were immunoblotted with antibodies for either AIB1, BRCA1, or actin.

Figure 4

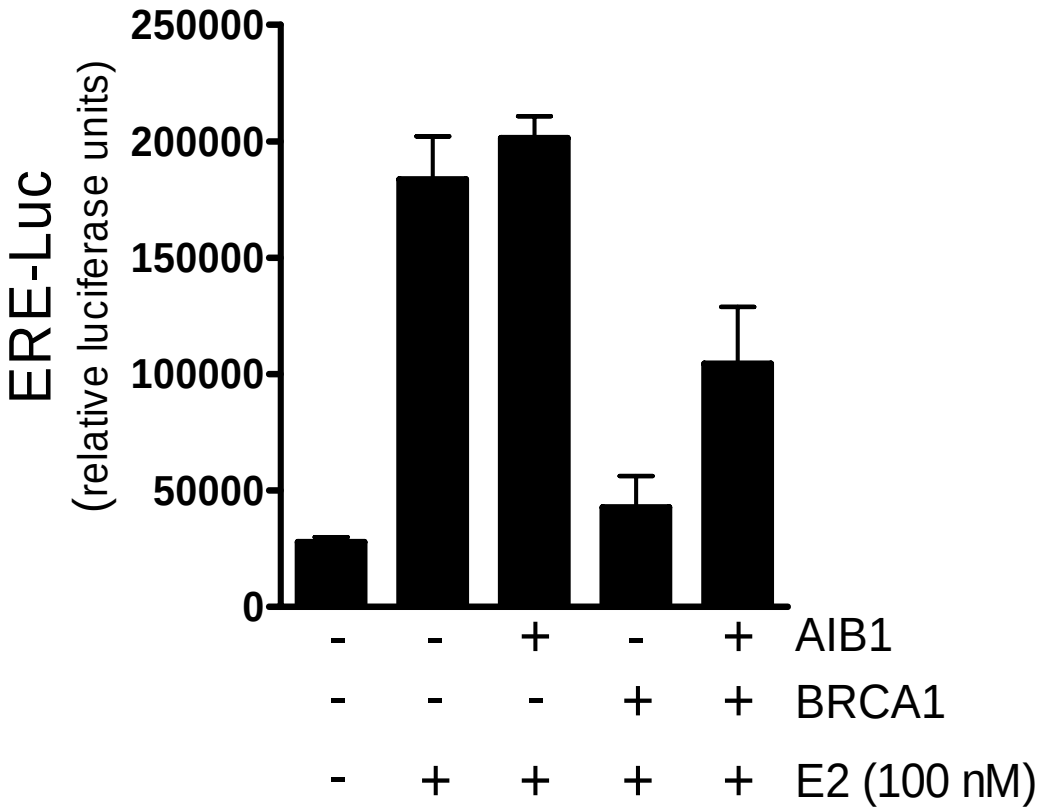


Figure 4. **Effect of AIB1 and BRCA1 on ERα transcriptional activity** MCF-7 cells were plated in 5% charcoal-stripped serum in IMEM for 3 days prior to transfection. Cells were transfected with the following plasmids: pcDNA3.1, pcDNA3-AIB1, and pcDNA3-BRCA1. The ERE reporter construct was cotransfected, which consists of 3 copies of the estrogen responsive element regulating luciferase. After 24 hrs of transfection, 100 nM of estrogen (E2) was added to the cells for another 24 hrs. The cells were lysed and luciferase levels were quantified. Conditions are in triplicate.

Figure 5

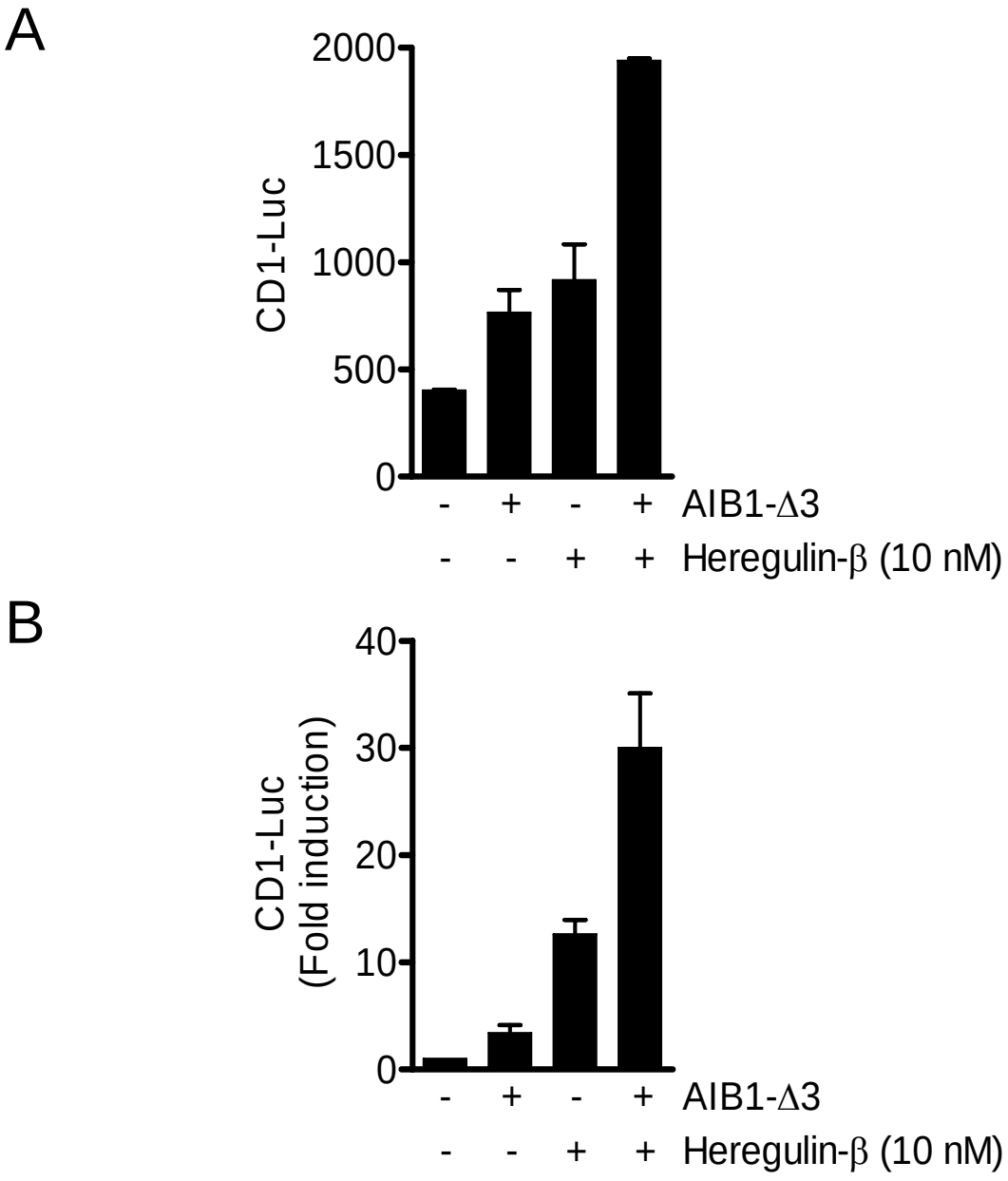


Figure 5. **AIB1-Δ3 enhances growth factor induced activation of the cyclin D1 promoter in HCC1937 cells (BRCA1-deficient) and MCF-7 cells (BRCA1-wild type)**. (A) HCC1937 and (B) MCF-7 cells were plated in IMEM containing 10% fetal bovine serum. After the cells were attached, the media was changed to serum-free IMEM. The cells were transfected with a -1745 cyclin D1 promoter-luciferase (CD1-Luc) reporter construct. After 24 hrs of transfection, heregulin-β (10 nM) was added to the cells. The cells were lysed after 24 hrs and luciferase activity was measured. The data from panel A represents a single experiment from triplicate samples. The data from panel B is from 5 separate experiments with 5 replicates per treatment and is represented as fold induction.

Figure 6

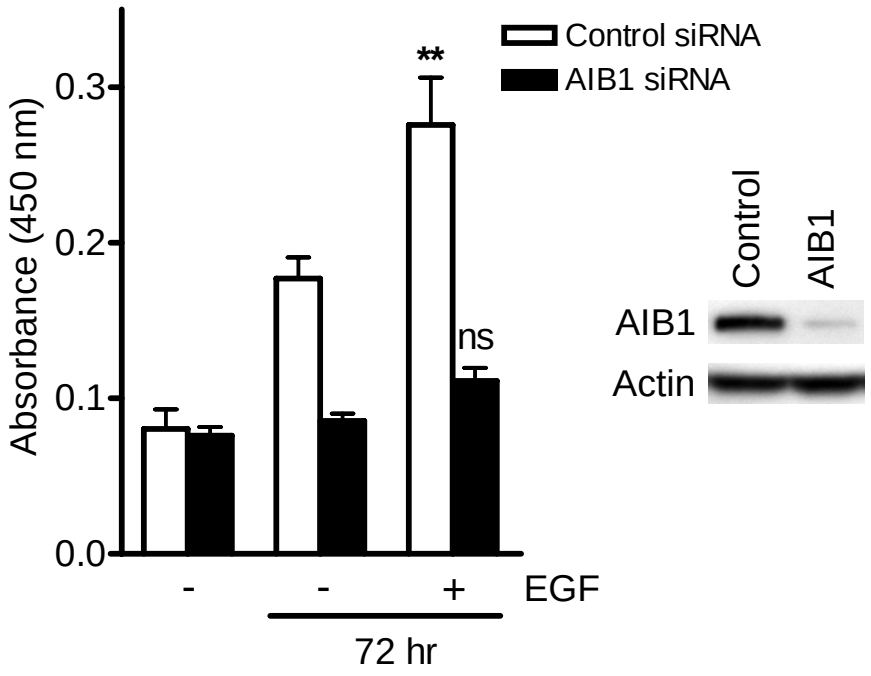


Figure 6. **AIB1 regulates EGF-stimulated proliferation of MDA-MB-231 breast cancer cells**.
MDA-MB-231 cells were transfected with either control siRNA (white bars) or AIB1 siRNA (black bars) for 24 hrs, seeded into 96-well plates at a density of 2,500 cells/well and then serum-starved for 24 hrs followed by EGF (50 ng/mL) treatment for 72 hrs. Cell proliferation was measured using the WST-1 assay. The inset shows the level of AIB1 protein expression by immunoblot after 4 days of siRNA treatment. The columns represent the mean ± SD of triplicate values from four independent experiments, ** $P<0.001$ (one-way ANOVA) relative to control siRNA results for each respective treatment group.

Figure 7

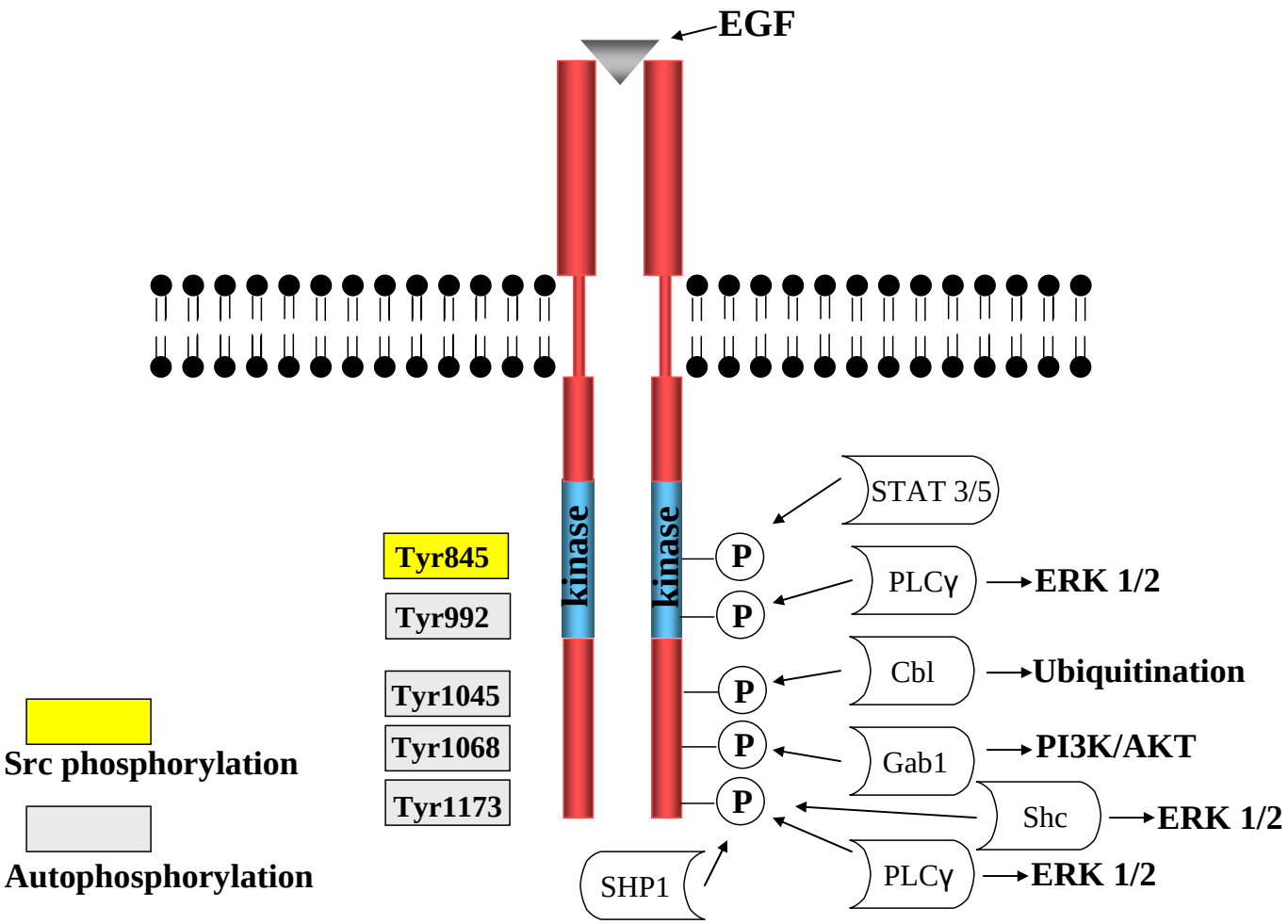
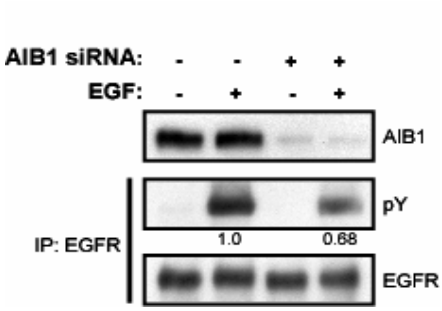


Figure 7. Diagram of EGFR phosphorylation sites and its interacting proteins.

Figure 8

A



B

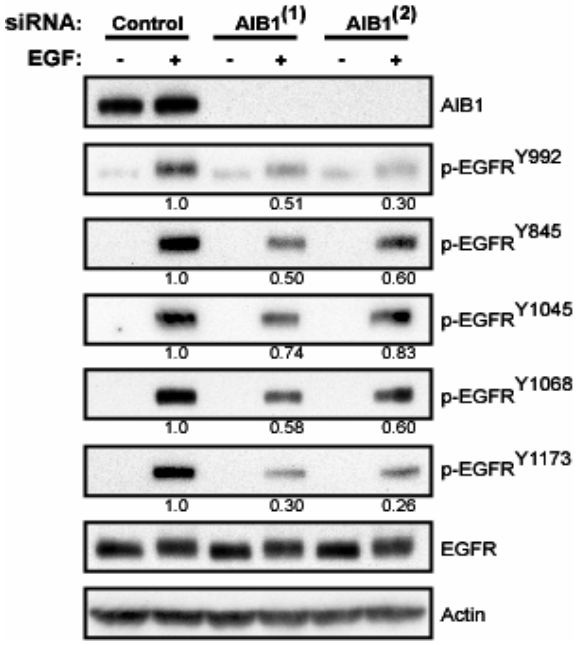


Figure 8. **Knockdown of AIB1 with AIB1 siRNA results in decreased phosphorylation of the EGF receptor in MDA-MB-231 breast cancer cells**. (A) MDA-MB-231 cells were treated with either control or AIB1 (1 & 2) siRNA for 24 hrs, serum-starved for 24 hrs, and then stimulated with 50 ng/ml of EGF for 10 min. Whole cell lysates were immunoprecipitated with anti-EGFR (528, Santa Cruz) and then immunoprecipitates were immunoblotted with either anti-phosphotyrosine (4G10, Millipore) or anti-EGFR (1005, Santa Cruz). AIB1 levels were detected with anti-AIB1 (BD Transduction). (B) MDA-MB-231 cells were treated as in panel A. Whole cell lysates were immunoblotted with phospho-specific EGFR antibodies (Cell Signaling) and total EGFR (1005, Santa Cruz).

Figure 9

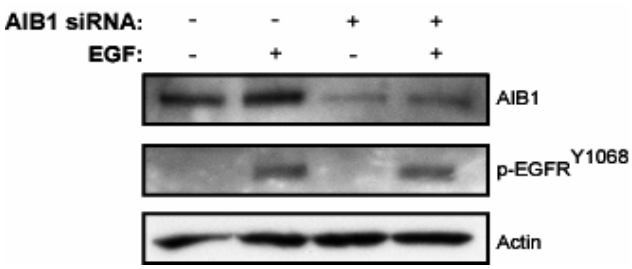
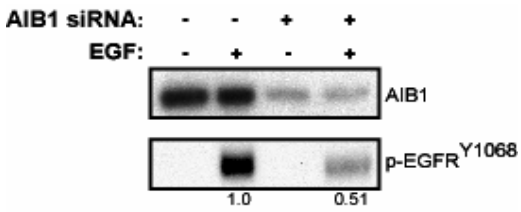


Figure 9. **Knockdown of AIB1 in HCC1937 breast cancer cells (BRCA1-deficient) does not affect phosphorylation of EGFR.** HCC1937 cells were transfected with either control or AIB1 siRNA for 24 hrs. The media was changed to serum-free media for 24 hrs. The cells were stimulated with EGF for 10 min. EGFR phosphorylation was assessed by immunoblot.

Figure 10

A



B

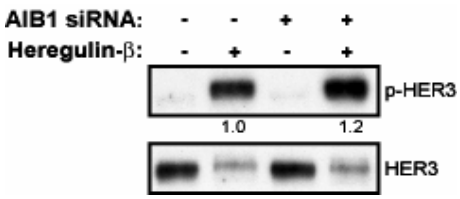


Figure 10. **Knockdown of AIB1 does not affect the phosphorylation of the EGFR family member, HER3.**
SK-BR-3 breast cancer cells were transfected with either control or AIB1 siRNA for 24 hrs. The media was changed to serum-free media for 24 hrs. The cells were stimulated with either EGF or heregulin-β (20 ng/ml) for 10 min. Activation of EGFR (A) and HER3 (B) was measured by immunoblot with phospho-specific antibodies to EGFR and HER3.

Figure 11

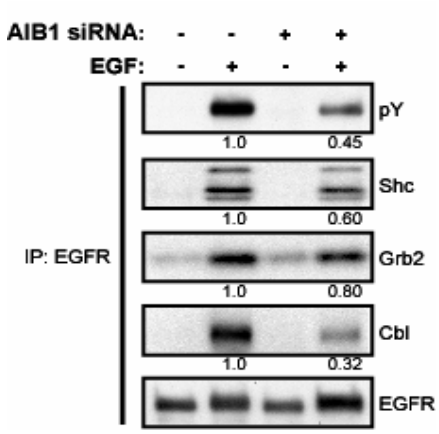


Figure 11. **Effect of reduced AIB1 protein levels on the recruitment of EGFR-interacting proteins.**
MDA-MB-231 cells were transfected with either control or AIB1 siRNA for 24 hrs. The media was changed to serum-free media for 24 hrs. The cells were stimulated with EGF for 10 min. EGFR was immunoprecipitated and interacting proteins were detected by immunoblot.

Figure 12

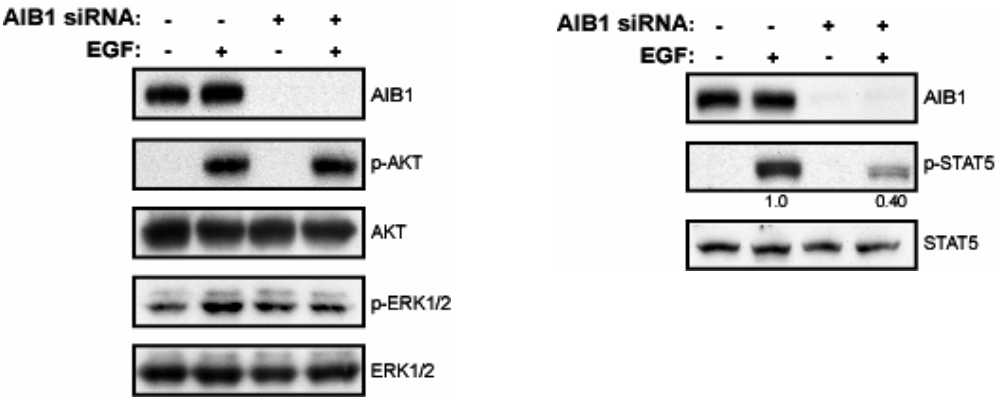


Figure 12. **Effect of reduced AIB1 protein levels on activation of downstream EGFR pathways.**
MDA-MB-231 cells were transfected with either control or AIB1 siRNA for 24 hrs. The media was changed to serum-free media for 24 hrs. The cells were stimulated with EGF for 10 min. Activation of AKT, ERK1/2, and STAT5 were assessed by immunoblot.

Figure 13

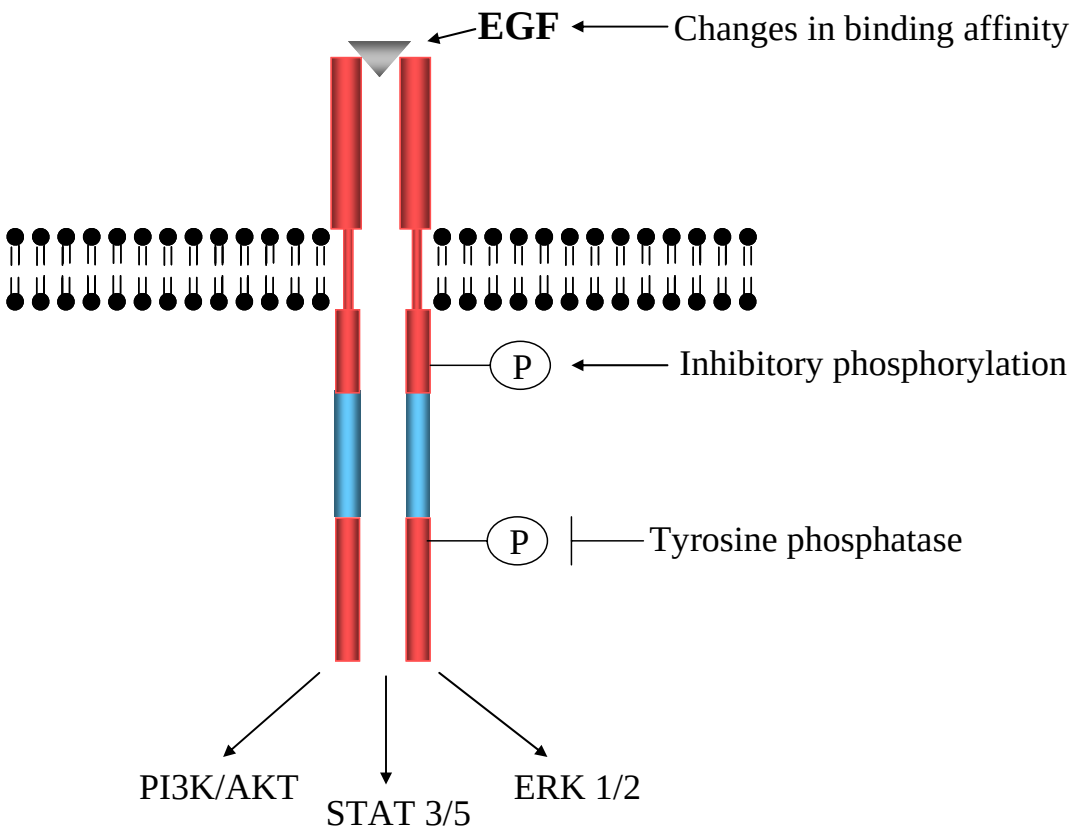


Figure 13. Model of possible mechanisms for the regulation of EGFR phosphorylation by AIB1.

Figure 14

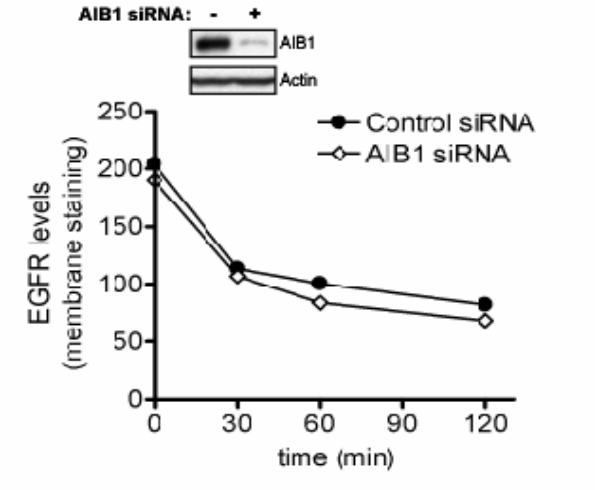
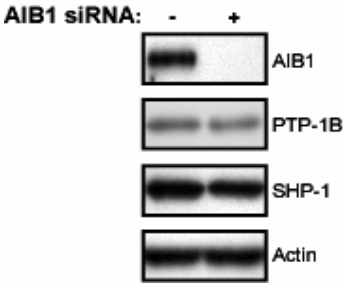


Figure 14. **Effect of reduced AIB1 protein levels on EGFR cell surface levels.**
MDA-MB-231 cells were treated with either control or AIB1 siRNA for 24 hrs. The media was changed to serum-free media for 24 hrs. The cells were stimulated with EGF from 30 to 120 min. EGFR cell surface levels were detected by flow cytometry analysis.

Figure 15

A



B

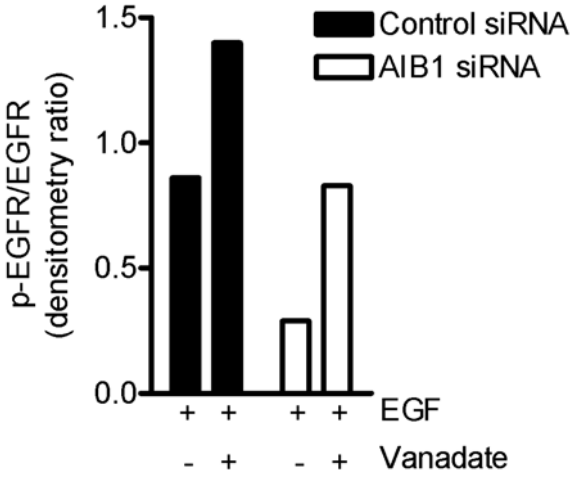


Figure 15. **The effect of AIB1 knockdown on the expression and/or activity of tyrosine phosphatases.**
MDA-MB-231 breast cancer cells were transfected with either control or AIB1 siRNA for 24 hrs. (A) The expression of the tyrosine phosphatases, PTP-1B and SHP-1, was determined by immunoblot. (B) The media was changed to serum-free media for 24 hrs and pre-treated with vanadate for 1 hr. The cells were stimulated with EGF for 10 min. Phosphorylation of EGFR was assessed by immunoblot. The densitometry values of phospho-EGFR over total EGFR levels is represented as a bar graph.

KEY RESEARCH ACCOMPLISHMENTS

- Growth factor induced cyclin D1 promoter activity is enhanced in both BRCA1-wild type and BRCA1-deficient breast cancer cells.
- AIB1 regulates the level of BRCA1 protein.
- A reduction in AIB1 expression inhibits the proliferation of BRCA1-wild type breast cancer cells.
- A reduction in AIB1 expression decreases EGF-induced activation of EGF receptor in BRCA1-wild type breast cancer cells but not in BRCA1-deficient breast cancer cells.
- A reduction in AIB1 expression decreases EGF-induced activation of ERK1/2 and STAT5.
- The effect of AIB1 knockdown on EGFR tyrosine phosphorylation is partially dependent on the activity of a tyrosine phosphatase.

REPORTABLE OUTCOMES

Future training in breast cancer research:

Postdoctoral fellowship at the National Institutes of Health/NIDDK/Mammalian Genetics Branch in the lab of Dr. Chuxia Deng. April 2008-April 2010.

Project: Signal transduction pathways that are involved in BRCA1-associated breast cancer.

Degrees:

Ph. D. degree in Tumor Biology obtained April 2007 from Georgetown University.

Publications:

1. **Lahusen T**, Wellstein A, Riegel AT. Role of AIB1 in hormone-independent breast cancer and anti-estrogen resistance. *Breast Cancer Research and Treatment* (Review) Submitted February 2008.

2. Oh A, **Lahusen T**, Chen C, Fereshteh M, Zhang X, Kagan B, Dakshanamurthy S, Xu J, Wellstein A, Riegel AT. Tyrosine Phosphorylation of the nuclear receptor coactivator

AIB1/SRC-3 is enhanced by Abl kinase and is required for its activity in cancer cells. *Mol. Cell. Biol.* Submitted February 2008.

3. **Lahusen T**, Fereshteh M, Oh A, Wellstein A, Riegel AT (2007) EGFR tyrosine phosphorylation and signaling controlled by a nuclear receptor coactivator AIB1. *Cancer Research*. 67(15):7256-652.

4. Mani A, Oh A, Bowden ET, **Lahusen T**, Lorick KL, Weissman AM, Schlegel R, Wellstein A, Riegel AT (2006) E6AP mediates regulated proteosomal degradation of the nuclear receptor coactivator amplified in breast cancer 1/SRC-3 in immortalized cells. Cancer Research. 66 (17):8680-6.

Abstracts:

1. **Lahusen JT**, Wellstein A, Riegel AT. AIB1 regulates EGFR phosphorylation and activity in cancer. Biomedical Sciences Research Fair (2006) Georgetown University, Washington, D.C.

2. Oh A, Stoica GE, **Lahusen JT**, Wellstein A, Riegel AT. Functional role of tyrosine phosphorylated AIB1/ACTR. Abstract No. 243. Keystone Symposia (2006) Nuclear Receptor: Orphan Brothers. Banff, Alberta, Canada.

CONCLUSIONS

From **Task 1**, it is unclear if AIB1 and BRCA1 interact due to high non-specific binding of AIB1 with the IgG immunoprecipitates. This is a technical limitation of the experiment. In addition, interaction of AIB1 and BRCA1 could not be detected in 293T cells transfected with Flag-tagged AIB1 and AIB1-Δ3. Also, as part of **Task 1**, preliminary experiments showed that AIB1 was able to partially reverse BRCA1-mediated suppression of ERα transcriptional activity in MCF-7 breast cancer cells. However, these results were not consistently reproducible, which will require further experimentation. A novel finding that AIB1 regulates the protein expression of BRCA1 was discovered from these studies. The mechanism for this regulation is unknown.

To address **Task 2**, AIB1-Δ3 and growth factors were shown to enhance cyclin D1 promoter activity, which could be used as a model system for testing the functional relationship of AIB1 and BRCA1 in growth factor-dependent gene expression. It was observed that induction of cyclin D1 promoter activity by both heregulin-β and AIB1-Δ3 was independent of the BRCA1 status of the cell. Additionally, AIB1 was shown to regulate EGF-stimulated proliferation of BRCA1-wild type MDA-MB-231 breast cancer cells. This was as a result of decreased EGF receptor tyrosine phosphorylation caused by a reduction in AIB1 levels with siRNA, which can be partially attributed to an increase in tyrosine phosphatase activity. Therefore, AIB1 can regulate the activity of EGFR in breast cancer cells which may enhance cell proliferation and survival. However, since a reduction of AIB1 in HCC1937 BRCA1-deficient cells did not result in decreased EGFR phosphorylation, this effect may depend on the expression of wild-type BRCA1.

ABBREVIATIONS

AIB1- Amplified in breast cancer 1
bHLH- basic helix-loop-helix
BRCA1- Breast Cancer Susceptibility Protein 1
EGFR- Epidermal Growth Factor Receptor
EGF- Epidermal Growth Factor
ERα- Estrogen Receptor alpha
ERK1/2- Extracellular-signal Regulated Kinase 1/2
IGF-1- Insulin-like Growth Factor 1
PAS- Per-Arnt-Sim
PR- Progesterone Receptor
siRNA- small-interfering RNA
SRC- Steroid Receptor Coactivator

REFERENCES

1. Anzick, S. L., Kononen, J., Walker, R. L., Azorsa, D. O., Tanner, M. M., Guan, X. Y., Sauter, G., Kallioniemi, O. P., Trent, J. M., and Meltzer, P. S. AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. Science, *277:* 965-968, 1997.

2. List, H. J., Reiter, R., Singh, B., Wellstein, A., and Riegel, A. T. Expression of the nuclear coactivator AIB1 in normal and malignant breast tissue. Breast Cancer Res Treat, *68:* 21-28, 2001.

3. Bouras, T., Southey, M. C., and Venter, D. J. Overexpression of the steroid receptor coactivator AIB1 in breast cancer correlates with the absence of estrogen and progesterone receptors and positivity for p53 and HER2/neu. Cancer Res, *61:* 903-907, 2001.

4. Reiter, R., Wellstein, A., and Riegel, A. T. An isoform of the coactivator AIB1 that increases hormone and growth factor sensitivity is overexpressed in breast cancer. J Biol Chem, *276:* 39736-39741, 2001.

5. List, H. J., Lauritsen, K. J., Reiter, R., Powers, C., Wellstein, A., and Riegel, A. T. Ribozyme targeting demonstrates that the nuclear receptor coactivator AIB1 is a rate-limiting factor for estrogen-dependent growth of human MCF-7 breast cancer cells. J Biol Chem, *276:* 23763-23768, 2001.

6. Torres-Arzayus, M. I., Font de Mora, J., Yuan, J., Vazquez, F., Bronson, R., Rue, M., Sellers, W. R., and Brown, M. High tumor incidence and activation of the PI3K/AKT pathway in transgenic mice define AIB1 as an oncogene. Cancer Cell, *6:* 263-274, 2004.

7. Oh, A., List, H. J., Reiter, R., Mani, A., Zhang, Y., Gehan, E., Wellstein, A., and Riegel, A. T. The nuclear receptor coactivator AIB1 mediates insulin-like growth factor I-induced phenotypic changes in human breast cancer cells. Cancer Res, *64:* 8299-8308, 2004.

8. Kuang, S. Q., Liao, L., Zhang, H., Lee, A. V., O'Malley, B. W., and Xu, J. AIB1/SRC-3 deficiency affects insulin-like growth factor I signaling pathway and suppresses v-Ha-ras-induced breast cancer initiation and progression in mice. Cancer Res, *64:* 1875-1885, 2004.

9. Fan, S., Wang, J., Yuan, R., Ma, Y., Meng, Q., Erdos, M. R., Pestell, R. G., Yuan, F., Auborn, K. J., Goldberg, I. D., and Rosen, E. M. BRCA1 inhibition of estrogen receptor signaling in transfected cells. Science, *284:* 1354-1356, 1999.

10. Ma, Y., Katiyar, P., Jones, L. P., Fan, S., Zhang, Y., Furth, P. A., and Rosen, E. M. The breast cancer susceptibility gene BRCA1 regulates progesterone receptor signaling in mammary epithelial cells. Mol Endocrinol, *20:* 14-34, 2006.

11. Park, J. J., Irvine, R. A., Buchanan, G., Koh, S. S., Park, J. M., Tilley, W. D., Stallcup, M. R., Press, M. F., and Coetzee, G. A. Breast cancer susceptibility gene 1 (BRCAI) is a coactivator of the androgen receptor. Cancer Res, *60:* 5946-5949, 2000.

12. Altiok, S., Batt, D., Altiok, N., Papautsky, A., Downward, J., Roberts, T. M., and Avraham, H. Heregulin induces phosphorylation of BRCA1 through phosphatidylinositol 3-Kinase/AKT in breast cancer cells. J Biol Chem, *274:* 32274-32278, 1999.

13. Wiley, H. S., Walsh, B. J., and Lund, K. A. Global modulation of the epidermal growth factor receptor is triggered by occupancy of only a few receptors. Evidence for a binary regulatory system in normal human fibroblasts. J Biol Chem, *264:* 18912-18920, 1989.

14. Theroux, S. J., Latour, D. A., Stanley, K., Raden, D. L., and Davis, R. J. Signal transduction by the epidermal growth factor receptor is attenuated by a COOH-terminal domain serine phosphorylation site. J Biol Chem, *267:* 16620-16626, 1992.

15. Tenev, T., Keilhack, H., Tomic, S., Stoyanov, B., Stein-Gerlach, M., Lammers, R., Krivtsov, A. V., Ullrich, A., and Bohmer, F. D. Both SH2 domains are involved in interaction of SHP-1 with the epidermal growth factor receptor but cannot confer receptor-directed activity to SHP-1/SHP-2 chimera. J Biol Chem, *272:* 5966-5973, 1997.

16. Tran, K. T., Rusu, S. D., Satish, L., and Wells, A. Aging-related attenuation of EGF receptor signaling is mediated in part by increased protein tyrosine phosphatase activity. Exp Cell Res, *289:* 359-367, 2003.